

The genus *Pronura* (Collembola: Neanuridae) in South America, with descriptions of two new species and a barcode sequence for one of them

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The genus *Pronura* (Collembola: Neanuridae) in South America, with descriptions of two new species and a barcode sequence for one of them.

- In this paper two new species of *Pronura* from South America are described and illustrated. *Pronura gaucheri* sp. n. from French Guyana has setae A, B, C and D of the antennofrontal setal group present; Ocp of the ocular group is absent; and a single ordinary microseta is present close to the sensilla on dorso-external tubercles of abdominal segments I-III. *Pronura paraguayana* sp. n. from Paraguay has setae A, B and D in the antennofrontal setal group (C absent); Ocp of the ocular group is present; and two ordinary setae (including one macroseta) are present in the dorso-external setal group of abdominal segments I-III. In addition to the morphological descriptions, the mitochondrial cytochrome c-oxidase subunit I COI sequence (barcode) of *Pronura gaucheri* sp. n. is provided. This the first time that a Collembola species is described along with its barcode sequence.

Keywords: French Guyana - Paraguay - Neanurinae - DNA barcoding - COI.

INTRODUCTION

To date, the genus *Pronura* comprises 53 named species (Bellinger *et al.*, 2010). All are recorded from tropical regions, but while Africa and southeast Asia host a large number of species (Cassagnau, 1996; Deharveng & Bedos, 1993), few are known from the Australian, southwestern Asian and American regions. Actually, the only South American record of the genus is *Pronura amazonica*, described from Brazil by Cassagnau & Pereira de Oliveira (1990). In this contribution, we describe two new species, one from French Guyana and the other from Paraguay. We also provide, for the first time in a taxonomic description of Collembola, the barcode for one of the new species.

MATERIAL AND METHODS

This paper is based on material recently collected by Cyrille D'Haese during a CaFoTrop expedition in French Guyana and on material in the collections of the Muséum d'histoire naturelle de Genève. All specimens were cleared and mounted in Marc-André II or Hoyer's medium. Drawings were done with a Leica MLS2 microscope using a drawing tube.

Type material is deposited in the Muséum d'histoire naturelle de Genève (MHNG), Universidad Nacional Autónoma de México (UNAM) and Muséum National d'Histoire Naturelle de Paris (MNHN).

The terminology used in the text and tables follows Palacios-Vargas & Simón-Benito (2007) (partly derived from Deharveng, 1981, 1983) and D'Haese (2003). Abbreviations: Abd. I, first abdominal segment; Ant. IV, fourth antennal segment; setal group or tubercles: De, dorso-external, Di, dorso-internal, DL, dorso-lateral, L, lateral, VL, ventro-lateral; M, long macrosetae; m, microseta; Oca, anterior ocular setae; Ocm, median ocular setae; Ocp, posterior ocular setae; s, setae s (sensorial setae); S.g.d., dorsal guard sensillum of Ant. III; S.g.v., ventral guard sensillum of Ant. III.

DNA was extracted from a 95% ethanol preserved specimen using a Qiagen DNeasy tissue extraction kit (Digestion overnight and re-suspension in 120 μ l of the elution buffer). Amplification was carried out in 25 μ l volume reaction using Amersham Bioscience puReTaq Ready-To-Go PCR Beads. The thermocycler program consisted of an initial denaturing step at 94°C for 2 min, 5 amplification cycles with a 45°C annealing temperature (94°C for 40s, 45°C for 40s, 72°C for 1 min), 30 cycles with a 51°C annealing temperature, and a final step at 72°C for 5 min. PCR amplification and sequencing were carried out with primers designed by Cyrille D'Haese in the BoEM Lab specifically for Collembola: LCO1490col (5' – WYT CDA CWA AYC RYA ARG AYA TYG G - 3') and HCO2198col (5' – TAN ACY TCN GGR TGN CCR AAR AAT CA - 3')

RESULTS

Pronura Delamare Deboutville, 1953

TYPE SPECIES: *Pronura kilimandjarica* Delamare Deboutville, 1953

DIAGNOSIS: Neanurinae, Paleonurini. No pigment. Dorsal tubercles not or only poorly developed. Usually no reticulations nor tertiary granulations. Maxilla needle-like, mandible bidentate or tridentate. 2 + 2 unpigmented eyes, sometimes absent. Sensilla on Ant. IV subequal. Posterior tergites not fused. No cryptopygy. No additional sensorial setae on the lateral group of abdominal tergites. Di setae, at least Di1, shifted towards De on Abd. V. Tibiotarsi without tenent hairs, unguis without tooth.

Pronura gaucheri sp. n.

Figs 1-7

HOLOTYPE: MNHN-EA010001 (GUY007); female on slide; French Guyana, Nouragues Field Station, Saut Pararé, 04°02.299' N, 052°40.303' W, 72 m asl.; leaf litter at the bottom of a Lecitidaceae, sifted and extracted in a Berlese-Tullgren apparatus; 23-IV-2009; leg. C. D'Haese.

PARATYPES: MNHN-EA010002 (GUY007), except for one paratype in MHNG and one at UNAM; three females, one male, one preadult male, four juveniles, all on slides; French Guyana, Nouragues Field Station, Saut Pararé, 4°02.299' N, 052°40.303' W, 72 m asl.; leaf litter



FIG. 1

Specimen of *Pronura gaucheri* sp. n. alive. This specimen is from the GUY038 locality and was barcoded.

at the bottom of a Lecitidacae, sifted and extracted in a Berlese-Tullgren apparatus; 23-IV-2009; leg. C. D'Haese.

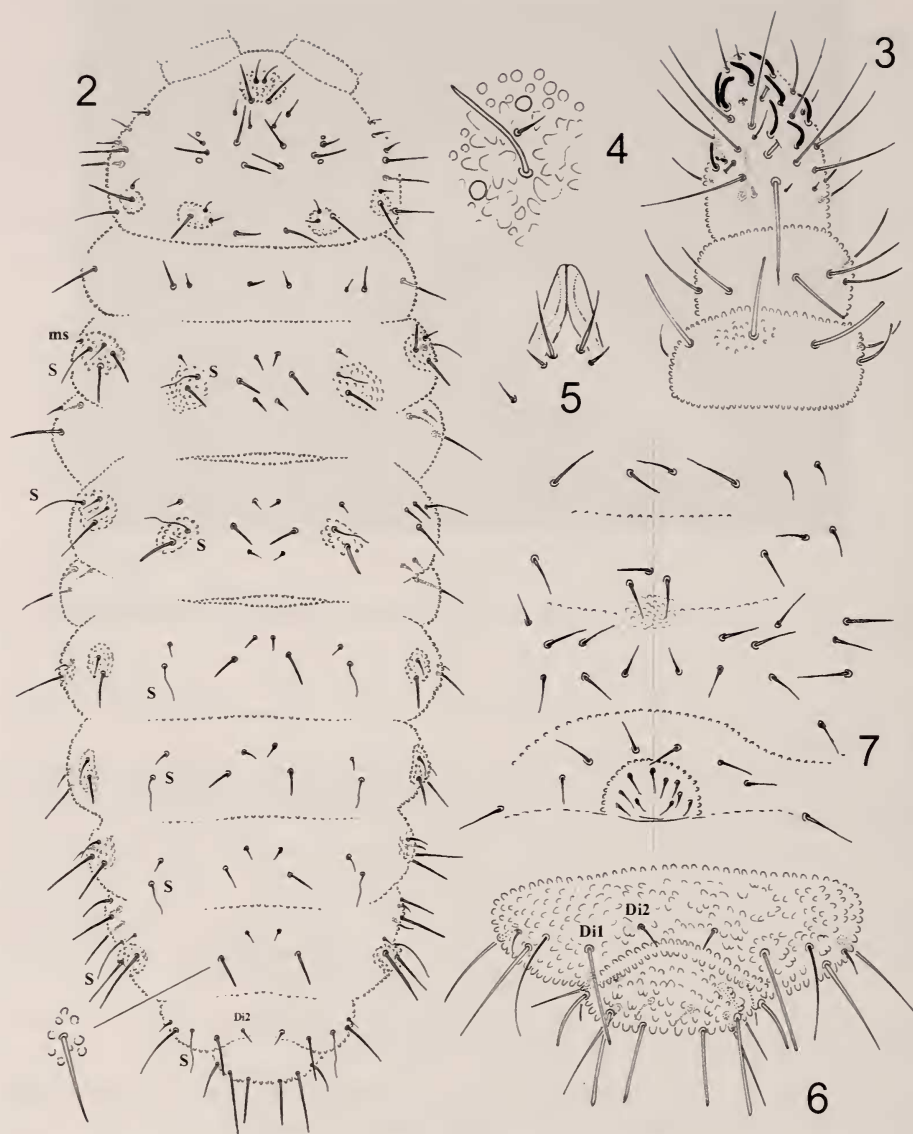
OTHER MATERIAL: GUY011, without registration numbers; 3 specimens; Saut Pararé, 4°02.293' N, 52°40.197' W, 64 m asl., dead branches and rotten log, sifted and extracted in a Berlese-Tullgren apparatus, CaFoTrop Expedition; 24-IV-2009; leg. D'Haese. – GUY035, without registration numbers; 2 specimens; French Guyana, Nouragues Field Station, Inselberg, 04°05.541' N, 052°40.646' W, 165 m asl. Leaf litter accumulation on the "plateau" at the base of the Inselberg, sifted and put on a Berlese-Tullgren apparatus CaFoTrop Expedition; 30-IV-2009; leg. D'Haese. – GUY038, 5 specimens (see Fig. 1), one voucher specimen deposited MNHN-EA010003; Inselberg. 4°05.793' N, 52°40.869' W, 407 m asl., summit forest, under bark and rotten logs, mouth aspirator, CaFoTrop Expedition; 01-V-2009; leg. D'Haese.

ETYMOLOGY: This new species is cordially dedicated to Philippe Gaucher, director of the Nouragues Field Station (CNRS-Guyane).

DIAGNOSIS: Head dorsally with seta C present, seta E absent and Ocp absent. One macroseta on De tubercles of Th. II-III, none on De of Abd. I-IV.

DESCRIPTION: Length ($n = 4$ adults) 0.59-0.94 mm. Color, white alive (Fig. 1). Secondary granules coarse; no tertiary granulation; tubercles indicated by coarser secondary granules, often hardly distinct on head, stronger but weak on De and DL of Th. II-III, and well developed on DL from Abd. I to III. Three kinds of ordinary dorsal setae: large smooth macrosetae being blunt apically and finely sheathed (M), medium-size smooth setae with blunt tips (mesosetae) and acuminate microsetae (m). Sensorial setae (s) long and thin, subequal or slightly longer than closest macrosetae (Fig. 2).

Ant. I with 7 setae, Ant. II with 11 setae. Ant. III sensorial organ with two globular sensilla in a cuticular fold, and two guard sensilla. S.g.v. almost straight and



FIGS 2-7

Pronura gaucheri sp. n., female holotype. (2) Dorsal chaetotaxy. (3) Antennal segments I-IV in dorsal view. (4) Left ocular area. (5) Labrum. (6) Abdominal tergites V and VI. (7) Chaetotaxy of abdominal sternites II-V.

bigger than s.g.d.; one ventro-external microsensillum. Ant. IV with 8 subequal sensilla (Fig. 3), twelve long and finely blunt setae (mou), one short acuminate seta (i) and a small subapical organ. No apical bulb differentiated.

Head with 6 indistinctly or very weakly delimited tubercles, marked by larger secondary granulation, its chaetotaxy as in Fig. 2. Eyes 2 + 2, unpigmented, very small, of same size as surrounding secondary granules (Fig. 4). Mandibles bidentate, maxillae styletiform. Labrum elongate, rounded apically (Fig. 5). Labrum formula ?0/2,4. Labium with 4 basal, 3 distal and 3 lateral setae, without conspicuous x-papillae, typical for most Neanurinae (e.g. fig. 4D in D'Haese, 2003). Five setae Vi on ventral side of head.

Tergite chaetotaxy as in Figs. 2 and Table 1. Abd. VI unilobed, with a single uneven, poorly differentiated tubercle (Fig. 6).

TABLE 1. Dorsal chaetotaxy of *Pronura gaucheri* sp. n.; tubercles inconspicuous on head. Setae number by setal groups.

A-Head chaetotaxy (by setal group)

Head setae group	Tubercles	Number of setae	Kind of setae	Setae
Cl+Af+Oc	±	16	8M, 8m	A, B, C, D, F, G, Oca, Ocm
Di	-	1	M	Di1
De	±	3	M, 2m	De1, Di2, De2
DL	±	3	2M, m	uncertain homologies
L+So	-	3	2M, 1m	uncertain homologies

B-Tergite chaetotaxy (by setal group on half tergites)

Thorax	DI	De	DL	L
I	me	M, m	M	-
II	M, 2m	M, m + s	2M, m + s + ms	M, 2m
III	M, 2m	M, m + s	M, 2m + s	M, 2m
Abdomen				
I	M, m	m + s	M, m	M, m
II	M, m	m + s	M, m	M, m
III	M, m	m + s	M, m	M, m
IV	M, m	2M, m + s		2M, 2 m
V		3M, 2m + s*		m
VI		(7+7)		

* Di2 mesoseta, not shift laterally, but clearly part of a (Di+De+DL) tubercle

Number of setae on legs I, II and III: tibiotarsi: 18, 18 and 17 (M absent); femora: 12-13, 11-12, 10; trochanters: 5, 5, 5; coxae: 3, 7, 8; subcoxae 2: 0, 1, 1. Unguis without tooth.

Ventral tube with 4 + 4 setae. Furcal vestige with 3 setae and no microseta (Fig. 7). Female genital plate with 3 + 3 pregenital, 8 circumgenital and 2 eugenital setae (Fig. 7). Male genital plate with 3 + 3 pregenital, 5 circumgenital and 4 + 4 eugenital setae. Anal lobes with 10-11 setae Ve; 2 microsetae An on each anal valve. No modified ventral setae in male.

TAXONOMIC REMARKS: *Pronura gaucheri* sp. n. is close to *P. amazonica*, the only species of the genus previously known from South America. *P. gaucheri* sp. n. differs mostly by the absence of macroseta De1 on Abd. I-III, tubercle (De+DL) posterior (versus internal) to L on Abd. IV, and 1 anterior mesoseta (versus 2) between axis and

sensilla of Abd. V. This last feature is also observed in the genus *Paramanura* Cassagnau, 1986, which includes a South-American species (*P. najtae* Cassagnau, 1986 from Venezuela, type species of the genus) and a Nepalese species (*P. problematica* Cassagnau, 1991). *P. gaucheri* differs from *Paramanura* taxa by the seta Di2 present and not shifted laterally on Abd. V, and by the dorso-external sensilla included in the (De+DL) tubercle of Abd. IV. The validity of the genus *Paramanura* has been questioned by Cassagnau himself (1991), and will be the subject of a further paper.

DNA BARCODE: A 658bp fragment of the COI gene has been amplified and sequenced from a specimen (Fig. 1) of the GUY038 locality. The sequence has been deposited into the Barcode of Life Database (BOLD) under accession number CDHO001-10 and in GenBank under the accession number JF411069.

***Pronura paraguayana* sp. n.**

Figs 8-14

HOLOTYPE: MHNG, without registration number; female on slide; Paraguay, Provincia Caaguazu, Río Yujury, 17 km south of Yhu, sifting in tropical dry forest; 9-IV-1985; leg. expédition zoologique du Muséum de Genève.

PARATYPES: MHNG, except for one paratype male in UNAM and one paratype female in MNHNP, without registration numbers; three females, two males, one preadult female, one juvenile, all on slides; collected together with the holotype.

OTHER MATERIAL: MHNG, without registration numbers; one female, three juvenils; Paraguay, Provincia Caaguazu, 20 km north of Coronel Oviedo (10 km south of Carayou), sifting of litter and dead trunks; 9-IV-1985; leg. expédition zoologique du Muséum de Genève.

ETYMOLOGY: The species name refers to the country of origin of the specimens examined (Paraguay).

DIAGNOSIS: Head dorsally with C absent, E absent and Ocp present. Two macrosetae (Th. II-III) and one macroseta (Abd. I-IV) on De tubercles of tergites.

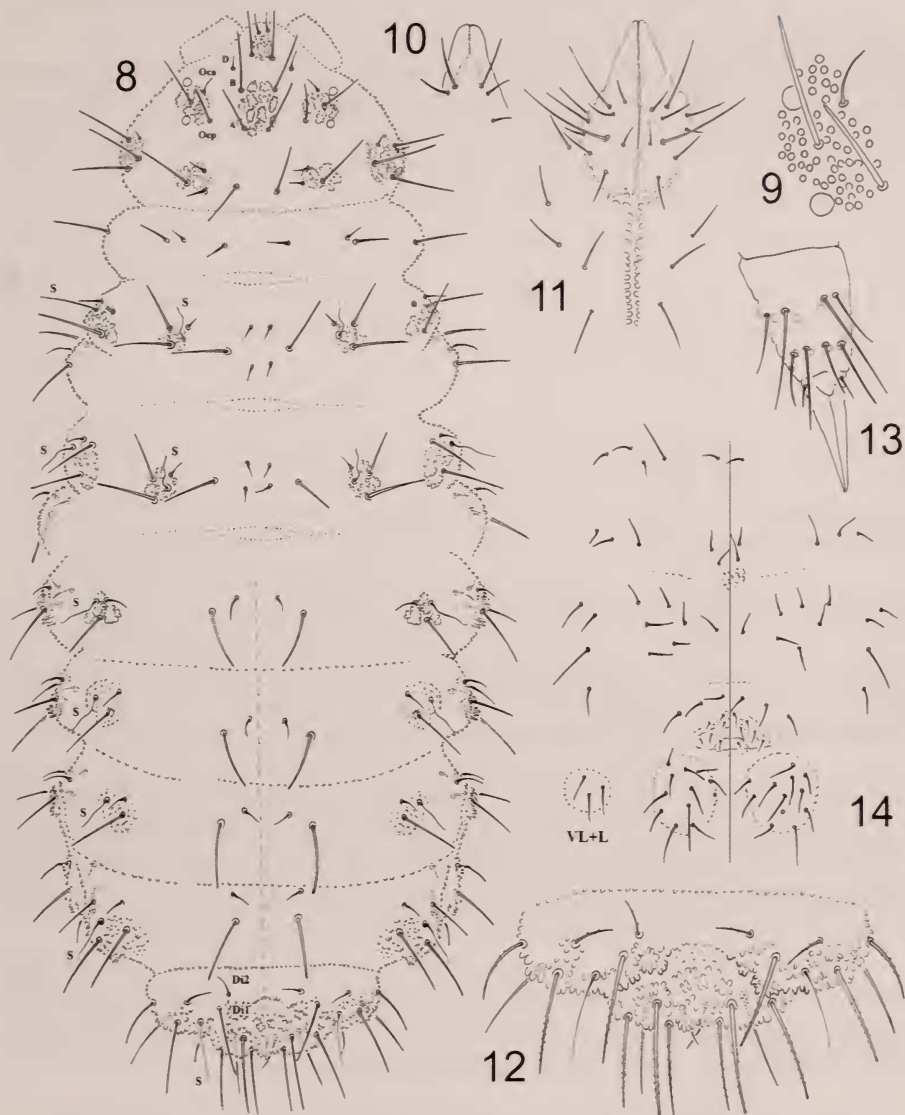
DESCRIPTION: Length (n = 6 adults) 0.76-1.08 mm. Color white. Granulation of the body fine, stronger on tubercles, these also indicated by tertiary granulation, mainly laterally and on Abd. V-VI. Tubercles De and DL developed, Di not developed from head to Abd. IV; tubercles of Abd. V-VI strongly developed. Two kinds of ordinary dorsal setae: thick, slightly barbate macrosetae (M) and shorter, thinner, more bent and less barbate microsetae (m). Sensorial setae (s) long and thin (Fig. 8).

Ant. I with 7 setae, Ant. II with probably 11 setae, the dorsal ones thicker and longer than the ventral ones. Ant. III sensorial organ with two globular sensilla in a cuticular fold, and two guard sensilla. S.g.v. almost straight and longer than S.g.d.; one ventral microsensillum. Ant. IV with 8 subequal sensilla, twelve long and finely blunt setae (mou), one short acuminate seta (i) and a small subapical organ. No apical bulb differentiated.

Eyes 2 + 2, unpigmented, large (diameter three times that of surrounding secondary granules) (Fig. 9). Mandibles with 2 teeth, maxillae styliiform. Head without well-developed tubercles. Labrum elongate, rounded apically (Fig. 10). Labrum formula ?/2,4. Labium with 4 basal, 3 distal and 3 lateral setae, without conspicuous x-papilla (Fig. 11).

Five cephalic tubercles (Table 2). Head chaetotaxy as in Fig. 8. Seta D free.

Tergite chaetotaxy as in Fig. 8 and Table 2. Tubercles of Abd. VI fused in a single, well differentiated tubercle (Fig. 12).



FIGS 8-14

Pronura paraguayana sp. n., female holotype. (8) Dorsal chaetotaxy. (9) Left ocular area. (10) Labrum. (11) Labium. (12) Abdominal segments V and VI. (13) Tibiotarsus of leg II in ventral view. (14) Chaetotaxy of abdominal sternites II-VI (group of setae VL+L surrounded with dotted line).

Number of setae on legs I, II and III: tibiotarsi: 18, 18 and 17 (M absent, Fig. 13); femora: ?12, ?, ?; trochanters: 6, 6, 6; coxae: 3, 7, 8; subcoxae 2: 0, 2, 2. Unguis without tooth. Tibiotarsi without tenent hairs (Fig. 13).

TABLE 2. Dorsal chaetotaxy of *Pronura paraguayana* sp. n. Setae number by setal groups.

A-Head chaetotaxy

Head setae group	Tubercles	Number of setae	Kind of setae	Setae
Cl	+	4	M, m	FG
Af	+	4	M	AB
	-	2	M	D
Oc	+	3	M	Oca, Ocm, Ocp
Di	-	1	M	Di1
De	+	3	M, m	De1, Di2, De2
DL+L+So	+	12	5M, 7m	uncertain homologies

B-Tergite chaetotaxy (by setal group on half tergites)

Thorax	DI	De	DL	L
I	M	M, m	M	-
II	M, 2m	2M, m + s	2M, m + s + ms	M, 2m
III	M, 2m	2M, m + s	2M, m + s	M, 2m
Abdomen				
I	M, m	M, m + s	M, m	M, 2m
II	M, m	M, m + s	M, m	M, 2m
III	M, m	M, m + s	M, m	M, 2m
IV	M, m		3M, m + s	5M, m
V		4M, 2m + s*		3m**
VI		(7+7)		

* Di2 mesoseta, shift laterally; ** including VL

Ventral tube with 4 + 4 setae. Furcal vestige with 4 setae and no microseta (Fig. 14). Female genital plate with 3+3 pregenital setae, 11-14 circumgenital setae and 2 eugenital setae (Fig. 14). Male genital plate with 3 + 3 pregenital setae, 10-12 circumgenital setae and 4 + 4 eugenital setae. Anal lobes with 12-13 setae Ve (Fig. 14); 2 microsetae An on each anal valve. No modified ventral setae in male.

TAXONOMIC REMARKS: *Pronura paraguayana* sp. n. differs from the other American species, *P. amazonica* and *P. gaucheri* sp. n., by different chaetotaxic characters (see Table 3), and by the morphology of its macrosetae (barbulate and of relatively longer size versus smooth and rather short).

TABLE 3. Comparative table of South-American species of *Pronura* and *Paramanura*

	Af	Oc	De Th. II-III	De Abd. I-III	De+DL Abd IV	L Abd I-II
<i>P. amazonica</i>	ABCD	Oca, Ocm	2+S	2+S	3+S	2
<i>P. gaucheri</i>	ABCD	Oca, Ocm	2+S	1+S	3+S	2
<i>P. paraguayana</i>	ABD	Oca, Ocm, Ocp	3+S	2+S	4+S	3
<i>Paramanura najtae</i>	ABD	Oca, Ocm	2+S	1+S	4+S	2

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